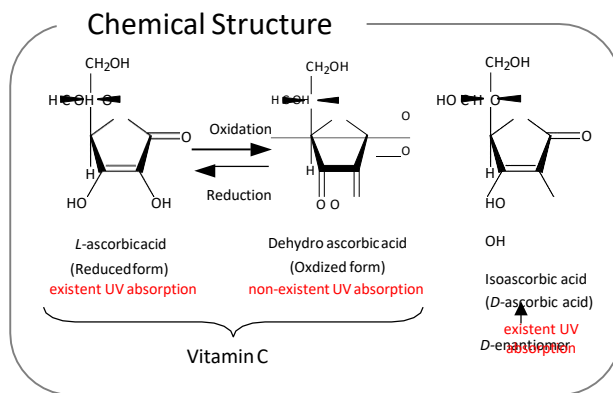


Analysis of Vitamin C in Food by HPLC

This is an application data of analyzing L-ascorbic acid and Dehydroascorbic acid, which are known to have a Vitamin C activity and Isoascorbic acid by HPLC using PDA.

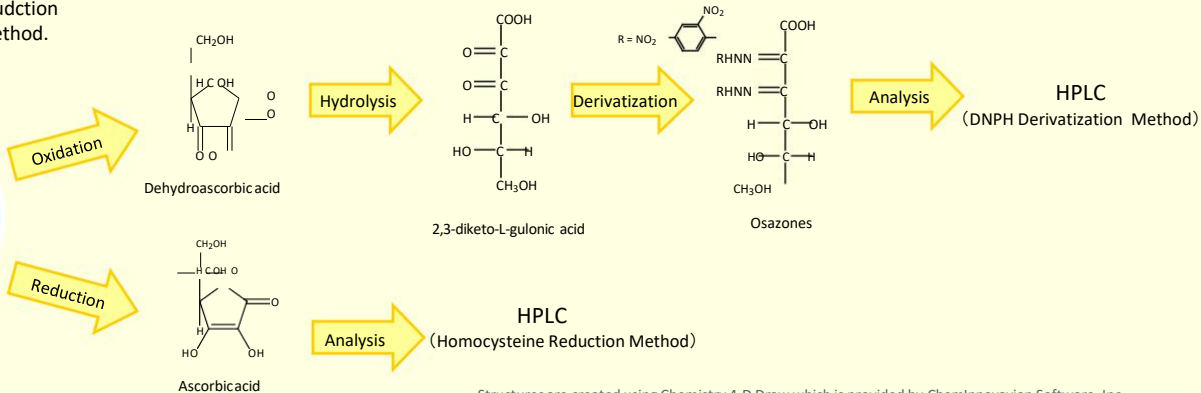
Dehydroascorbic acid is a Vitamin C compound like Ascorbic acid. Dehydroascorbic acid (DHAsA) is an oxidized form of Ascorbic acid (AsA). AsA can be detected by an UV Detector, but DHAsA can not. Therefore, it is necessary to convert the structure of the compound to make it detected by an UV Detector analyzing the total amount of Vitamin C. Also, there is an isomer of AsA known as Isoascorbic acid (ErA), which is a food additive.

This application was conducted based on the Japanese Food Sanitation Inspection Guideline.



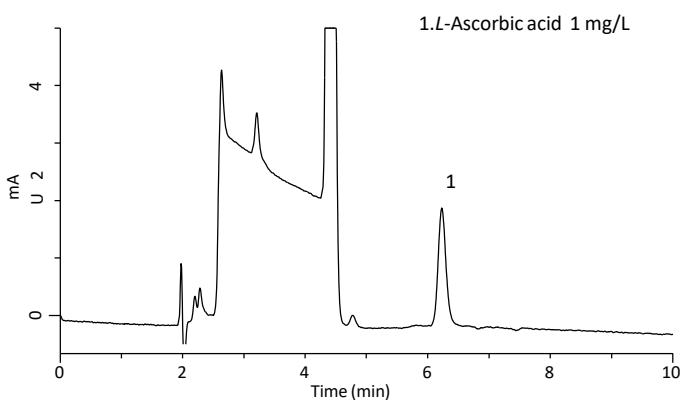
Outline

The total amount of Ascorbic acid can be measured by a DNPH Derivatization method. Simultaneous analysis of Isoascorbic acid and Reduced L-ascorbic acid can be measured by a Homocysteine reduction method.

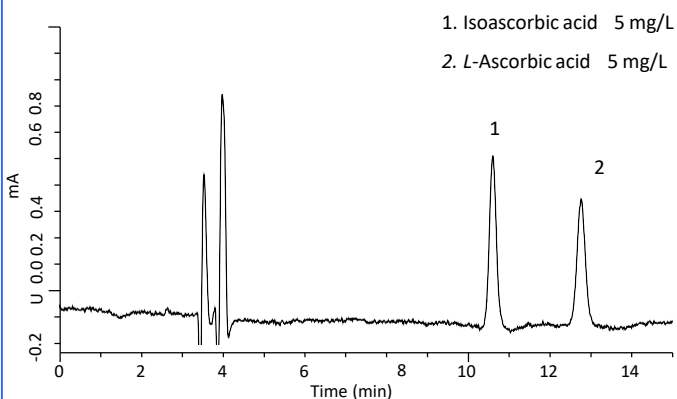


Analysis of Standard Solution

DNPH Derivatization Method



Homocysteine Reduction Method



Analytical Conditions ①

Column : Inertsil SIL-100A
(5μm, 250 x 4.6 mm I.D.)

Mobile Phase : A) CH₃COOC₂H₅
B) *n*-Hexane
C) CH₃COOH
A/B/C = 50/40/10, v/v/v

Flow Rate : 1.5 mL/min

Column Temp. : 40 °C

Detection : PDA 495 nm

Injection Volume : 20 μL

Analytical Conditions ②

Column : Inertsil NH₂
(5μm, 250 x 4.6 mm I.D.)

Mobile Phase : A) CH₃CN B) CH₃OH
C) 0.01M phosphoric Buffer
D) 0.03% homocystein solution
A/B/C/D = 600/30/100/30, v/v/v/v
: 1.0 mL/min

Flow Rate : 40 °C

Column Temp. : PDA 270 nm

Detection

Injection Volume : 5 μL

DNPH Derivatization Method

Pretreatment Conditions

Sample

- 5 g
- 5 %Metaphosphoric acid 30 mL
- grinding extraction
- Dilute to 50 mL with 5 % Metaphosphoric acid

Filtration

- Centrifugation 3000 rpm, 10 min
- 0.45 μm Filter

Fractionation

- 2mL Fraction

Derivatization

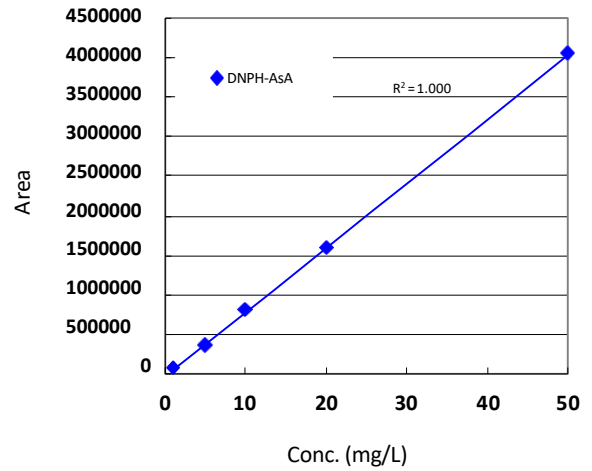
- 5 %Metaphosphoric acid 1 mL
- 2,6-dichloroindophenol 3 drop
- 2 %thiourea ·Metaphosphoric acid solution 2 mL
- 2 % 2,4-DNPH · 4.5M Sulfuric acid 0.5 mL
- Heating (50°C, 90 min)
- Water cooling

liquid-liquid extraction

- Ethyl acetate 2 mL
- Shake 1 hr

Measurement sample

- Supernatant liquid
 - 0.5 mL Fraction
 - Dilute to 1 mL with Hexane
- Lower layer
Waste

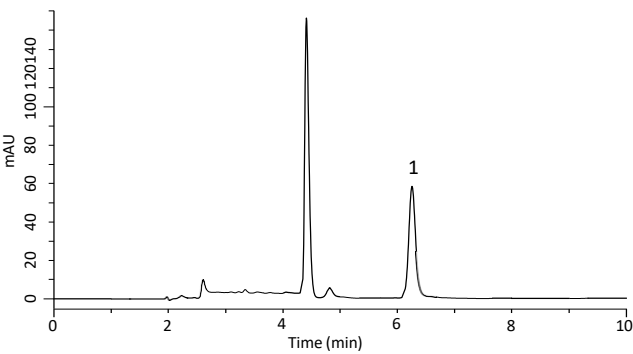


Calibration Curve*1

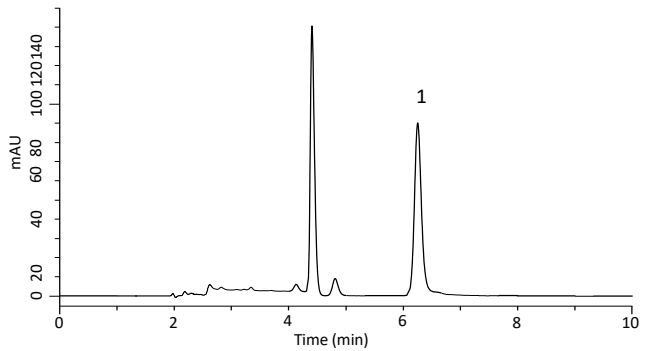
*1: The calibration sample was prepared by diluting L-Ascorbic acid in steps and pretreating it. The concentration described above is the concentration after diluting the sample.

Analysis of food (Analytical Conditions ①)

Tea leaf

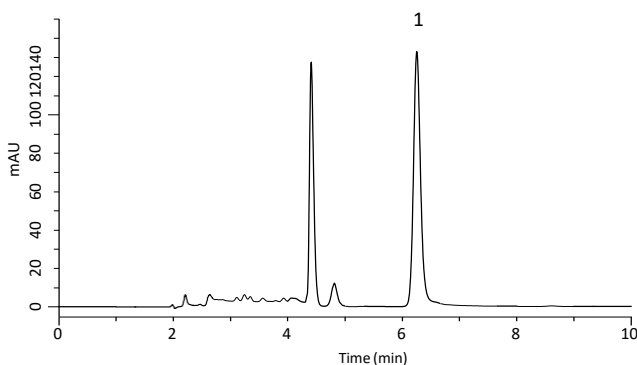


Sausage

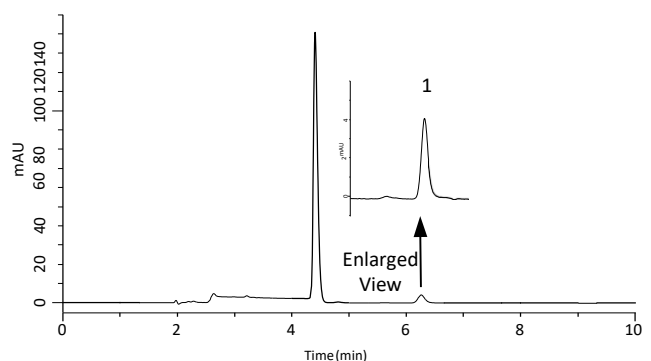


1. L-Ascorbic acid

Baby formula



Spinach



Liquid Sample

10 g
4 % Metaphosphoric acid 10 mL
Dilute to 50 mL
with 2 % Metaphosphoric acid

Solid Sample

10 g
4 % Metaphosphoric acid 10 mL
2 % Metaphosphoric acid 30 mL
ultrasonic extraction 10 min
Dilute to 50 mL
with 2 % Metaphosphoric acid

Filtration

Centrifugation 3000 rpm 10 min
0.45 μm Filter

2 mL Fraction

2 mL Fraction

Reduction

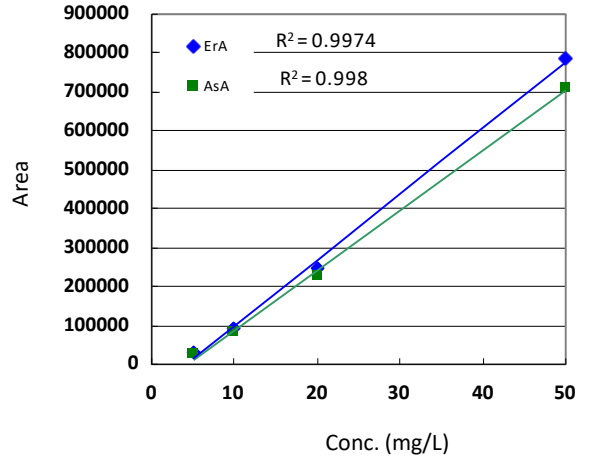
0.1 % Homocystein 1 mL
10 % Disodium Hydrogen Phosphate 1 mL
Heating (40 °C, 20 min)

Measurement

L-ascorbic acid
+
Iso ascorbic acid

Measurement

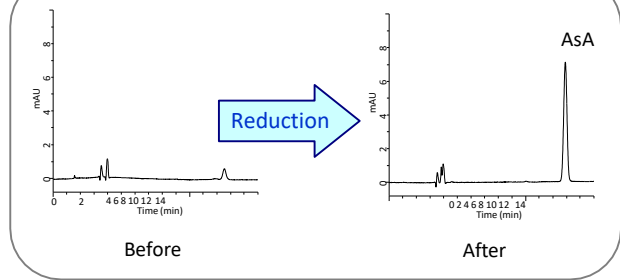
Total Ascorbic acid
+
Total Iso ascorbic acid



Calibration Curve*2

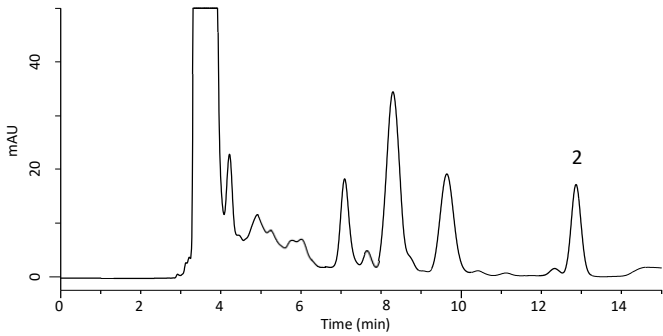
*2: On this figure, standard solution is diluted by 2 % Metaphosphoric acid.

Effect of Reduction Method

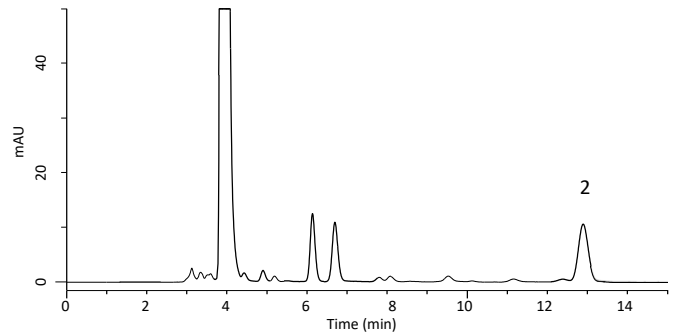


Analysis of food (Analytical Conditions ②)

Tea leaf

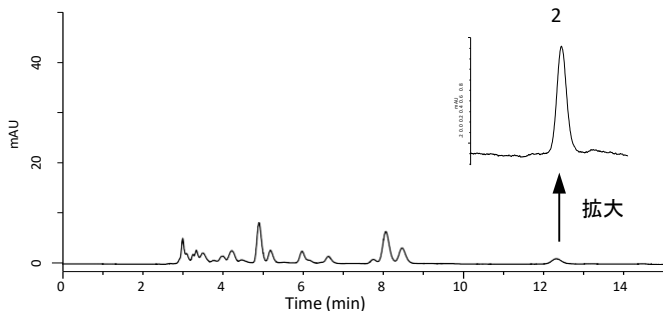


Sausage

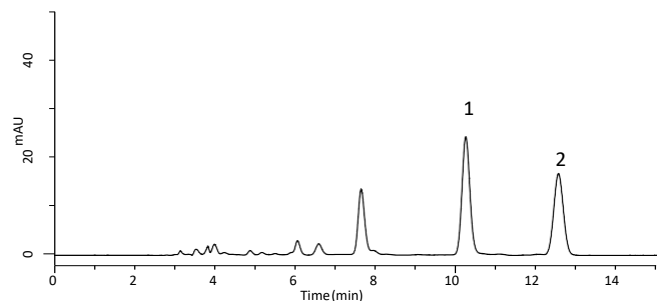


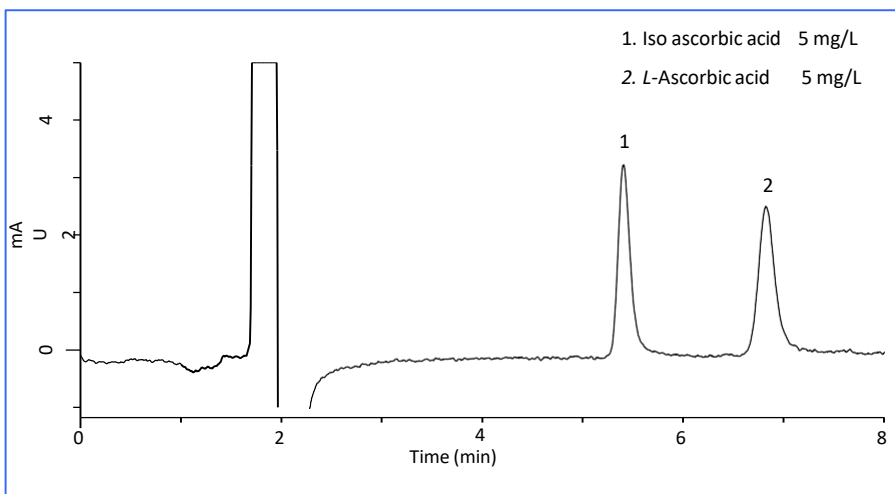
1. Iso ascorbic acid
2. L-Ascorbic acid

Beer



Fish sausage



Modified analytical conditions by Homocysteine Reduction Method**Analytical Conditions ③**

| | |
|-------------------------|---|
| Column | : Inertsil NH ₂ (5 μm, 250 x 4.6 mm I.D.) |
| Mobile Phase | : A) CH ₃ CN B) H ₂ O C) CH ₃ COOH A/B/C = 87/11/2, v/v/v |
| Flow Rate | : 2.0 mL/min |
| Column Temp. | : 40 °C |
| Detection | : PDA 243 nm |
| Injection Volume | : 20 μL |

GL Sciences disclaims any and all responsibility for any injury or damage which may be caused by this data directly or indirectly. We reserve the right to amend this information or data at any time and without any prior announcement.

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